New Application Preliminary Amendment Attorney Dockt: P69705US0
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Amendments to the Claims:

The listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

- 1. (original) A fusion polypeptide for expression in a host cell comprising a TolAIII domain or a functional homologue, fragment, or derivative thereof and a non-TolA polypeptide, wherein the TolAIII domain or functional homologue, fragment, or derivative thereof is located towards the N-terminus of the fusion polypeptide and the non-TolA polypeptide is located towards the C-terminus of the fusion polypeptide.
- 2. (original) The fusion polypeptide according to claim 1, further comprising a signal peptide.
- 3. (original) The fusion polypeptide according to claim 2, in which the signal peptide is located at or near the N-terminus of the fusion polypeptide.
- 4. (currently amended) The fusion polypeptide according to <u>claim 1</u> any preceding elaim, wherein the TolAIII domain or functional homologue, fragment, or derivative thereof has been codon-optimised for expression in the host cell.
- 5. (currently amended) The fusion polypeptide according to <u>claim 1</u> any of the <u>preceding claims</u>, further comprising a linker between the TolAIII domain or functional homologue, fragment, or derivative thereof and the non-TolA polypeptide.
- 6. (original) The fusion polypeptide according to claim 5, wherein the linker comprises at least one cleavage site for an endopeptidase.

- 7. (currently amended) The fusion polypeptide according to claim 6, wherein the cleavage site comprises the amino acid sequence of DDDDK (SEQ ID NO: 3) and/or LVPR (SEQ ID NO: 4) and/or IEGR (SEQ ID NO: 5).
- 8. (currently amended) The fusion polypeptide according to <u>claim 1</u> any of the <u>preceding claims</u>, further comprising an affinity purification tag.
- 9. (original) The fusion polypeptide according to claim 8, wherein the affinity purification tag is located at or near the N-terminus of the fusion polypeptide.
- 10. (original) The fusion polypeptide according to claim 9, wherein the affinity purification tag is an N-terminal His_n tag, with n=4, 5, 6, 7, 8, 9 or 10 (SEQ ID NOs: 6 12, respectively; preferably n=6 [SEQ ID NO: 8]), optionally with the His_n tag linked to the fusion polypeptide by one or more Ser residues (preferably 2).
- 11. (currently amended) The fusion polypeptide according to <u>claim 1 any of the preceding claims</u>, wherein the TolAIII domain consists of amino acid residues 329-421 of (SEQ ID NO: 13) of the *Escherichia coli* TolA sequence (SwissProt Acc. No. P19934).
- 12. (currently amended) The fusion polypeptide according to <u>claim 1 any of the preceding claims</u>, wherein the host cell is bacterial (for example, *Escherichia coli*).
- 13. (currently amended) The fusion polypeptide according to <u>claim 1 any of the preceding claims</u>, wherein the non-TolA polypeptide is BCL-XL.
- 14. (currently amended) A DNA molecule encoding the fusion polypeptide as defined in claim 1 any of claims 1-13.
- 15. (currently amended) <u>The A-DNA</u> molecule according to claim 14, wherein the mRNA properties of the DNA molecule when transcribed are optimised for expression in the host cell.

New Application Preliminary Amendment

16. (currently amended) An expression vector comprising the DNA molecule according to either of claim 14 or claim 15 for expression of the fusion polypeptide defined in any of claims 1-13.

- 17. (original) The expression vector according to claim 16, having an inducible promoter (for example, the IPTG-inducible T7 promotor) which drives expression of the fusion polypeptide.
- 18. (currently amended) The expression vector according to either of claim 16 or elaim 17, having an antibiotic resistance marker (for example, the *bla* gene, which confers resistance to ampicillin and chloramphenicol).
- 19. (currently amended) A cloning vector for producing the expression vector defined in <u>claim 16</u> any of claims 16-18, comprising DNA encoding the TolAHI domain or a functional homologue, fragment, or derivative thereof upstream or downstream from a cloning site which allows in-frame insertion of DNA encoding a non-TolA polypeptide.
- 20. (currently amended) The cloning vector according to claim 19, further comprising DNA encoding at least one cleavage site (for example, the amino acid sequence of DDDDK [SEQ ID NO: 3] and/or LVPR [SEQ ID NO: 4] and/or IEGR [SEQ ID NO: 5]) for an endopeptidase, the cleavage site located between the DNA encoding the TolAIII domain or a functional homologue, fragment, or derivative thereof and the cloning site.
- 21. (currently amended) The cloning vector according to <u>claim 19 either of claims 19</u> or 20, wherein the cloning site comprises at least one restriction endonuclease (for example, *Bam*HI and/or *Kpn*I) target sequence.
- 22. (currently amended) The cloning vector according to <u>claim 19</u> any of claims 19-21, further comprising DNA encoding an affinity purification tag as defined in either of <u>claim 8 or claim 9</u>.

- 23. (currently amended) The cloning vector according to <u>claim 19</u> any of claims 19-22, further comprising an inducible promoter (for example, the IPTG-inducible T7 promotor).
- 24. (currently amended) The cloning vector according to <u>claim 19</u> any of claims 19-23, further comprising DNA encoding an antibiotic resistance marker (for example, the *bla* gene, which confers resistance to ampicillin and chloramphenicol).
- 25. (currently amended) The cloning vector according to <u>claim 19</u> any of claims 19-24, having the structure of pTolE, pTolT or pTolX (as shown in Figure 2 with reference to the description).
- 26. (currently amended) Use of the TolAIII domain or functional homologue, fragment, or derivative thereof for production of a fusion polypeptide as defined in <u>claim</u> 1 any of claims 1-13.
- 27. (currently amended) Use of the TolAIII domain or functional homologue, fragment, or derivative thereof for production of the DNA molecule as defined in <u>claim</u> 14 either of claim 14 or claim 15.
- 28. (currently amended) Use of the TolAIII domain or functional homologue, fragment, or derivative thereof for production of an expression vector as defined in <u>claim</u> 16 any of claims 16-18.
- 29. (currently amended) Use of the TolAIII domain or functional homologue, fragment, or derivative thereof for production of a cloning vector as defined claim 19 in any of claims 19-25.
- 30. (currently amended) A host cell containing the DNA as defined in claim 14 13 and/or an the expression vector comprising the DNA molecule for expression of the fusion peptide as defined in any of claims 16-18 and/or an the cloning vector for producing the expression vector which comprises DNA encoding the TolAIII domain or a

functional homologue, fragment, or derivative thereof upstream or downstream from a cloning site which allows in-frame insertion of DNA encoding a non-TolA polypeptide as defined in any of claims 19-25.

- 31. (currently amended) Use of the fusion polypeptide as defined in <u>claim 5 any of elaims 5-13-for</u> immobilisation of the non-TolA polypeptide, comprising the step of: binding the fusion polypeptide to a TolA binding polypeptide (e.g. the TolA-recognition site of colicin N or other colicins, the TolA binding region of bacteriophage g3p-D1 protein, or the TolA binding region of TolB or other Tol proteins).
- 32. (currently amended) Use of the fusion polypeptide as defined in <u>claim 9 any of elaims 9-13</u>-for immobilisation of the non-TolA polypeptide, comprising the step of: binding the affinity tag of the fusion polypeptide to a binding moiety.
- 33. (currently amended) Use of the fusion polypeptide as defined in <u>claim 5 any of elaims 5-13</u> for purification and isolation of the non-TolA polypeptide, comprising the steps of:
- (i) binding the fusion polypeptide to a TolA binding polypeptide (e.g. the TolA-recognition site of colicin N or other colicins, the TolA binding region of bacteriophage g3p-D1 protein, or the TolA binding region of TolB or other Tol proteins);
- (ii) cleaving the non-TolA polypeptide from the TolAIII domain or functional homologue, fragment, or derivative thereof using an endopeptidase; and
- (iii) separating the cleaved non-TolA polypeptide from the TolAIII domain or functional homologue, fragment, or derivative thereof.
- 34. (currently amended) Use of the fusion polypeptide as defined in <u>claim 8 any of elaims 8-13</u> for purification and isolation of the non-TolA polypeptide, comprising the steps of:
- (i) binding the affinity tag of the fusion polypeptide to a binding moiety;
- (ii) cleaving the non-TolA polypeptide from the TolAIII domain or functional homologue, fragment, or derivative thereof using an endopeptidase; and

New Application Preliminary Amendment

(iii) separating the cleaved non-TolA polypeptide from the TolAIII domain or functional homologue, fragment, or derivative thereof.

- 35. (currently amended) Use of the fusion polypeptide as defined in <u>claim 1 any of elaims 1-13</u> for studying interaction properties of the non-TolA polypeptide or the fusion polypeptide, for example self-interaction, interaction with another molecule, or interaction with a physical stimulus.
- 36. (currently amended) A method for high expression of a polypeptide as a fusion polypeptide in a host cell, comprising the step of expressing the polypeptide as a fusion polypeptide as defined in <u>claim 1 any of claims 1-13</u> in a host cell.